Clinical Molecular Profiling Core

Developing a New Laboratory Paradigm in Clinical Research Care

Daniel C. Edelman, Ph.D.
Genetics Branch
June 12th, 2008

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CENTER FOR CANCER RESEARCH

Sound Bites

Gene Expression Signatures, Clinicopathological Features, and Individualized Therapy in Breast Cancer

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Context Gene expression profiling may be useful for prognostic and t
egies in breast carcinoma.

Objectives To demonstrate the value in integrating genomic inform
cal and pathological risk factors, to refine prognosis, and to improve th
egies for early stage breast cancer.

Design, Setting, and Patients Retrospective study of patients v
breast carcinoma who were candidates for adjuvant chemotherapy; 9
notated breast tumor samples (573 in the initial discovery set and 3
tion cohort) with corresponding microarray data were used. All pa
signed relapse risk scores based on their respective clinicopathological fea
representing oncogenic pathway activation and tumor biology (microe
tus were applied to these samples to obtain patterns of deregulation
with relapse risk scores to refine prognosis with the clinicopathological p
alone. Predictors of chemotherapeutic response were also applied to
terize clinically relevant heterogeneity in early stage breast cancer.

Main Outcome Measures Gene expression signatures and clinicop
ables in early stage breast cancer to determine a refined estimation of r
vival and sensitivity to chemotherapy.

Results In the initial data set of 573 patients, prognostically significa
resenting patterns of oncogenic pathway activation and tu
microenvironment states were identified within the low-risk (log-rank
mediate-risk (log-rank $P = .01$), and high-risk (log-rank $P = .003$)
representing clinically important genomic subphenotypes of breast ca
ample, in the low-risk cohort, of 6 prognostically significant clusters,
ter 4 had an inferior relapse-free survival vs patients in cluster 1 (log
and cluster 5 (log-rank $P = .03$). Median relapse-free survival for patie
was 16 months less than for patients in cluster 1 (95% CI, 7.5-24.5 m
months less than for patients in cluster 5 (95% CI, 10.5-27.5 month
analyses confirmed the independent prognostic value of the genomic cl
$P = .05$; high risk, $P = .02$). The reproducibility and validity of these pa
way deregulation in predicting relapse risk was established using relate
tical clusters in the independent validation cohort. The prognostic clinic
ters also have unique sensitivity patterns to commonly used cytotoxic

Conclusions These results provide preliminary evidence that incorporatio
sion signatures into clinical risk stratification can refine prognosis. Prospe
needed to determine the value of this approach for individualizing therap

JAMA. 2008;299(13):1574-1587

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man, Moylan, Mukherjee, Barry, Dressman, Ginsburg, Garman, Nevins, and Potti, Mr Acharya, and Ms Salter and Walters) and Institute for Statistics and Decision Sciences (Drs Mukherjee and Barry), Duke University; and Department of Medicine, Duke University Medical Center (Drs H
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JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

First-Line Gefitinib in Patients With Advanced Non–Small-Cell Lung Cancer Harboring Somatic *EGFR* Mutations

Lecia V. Sequist, Renato G. Martins, David Spigel, Steven M. Grunberg, Alexander Spira, Pasi A. Jänne, Victoria A. Joshi, David McCollum, Tracey L. Evans, Alona Muzikansky, Georgiana L. Kuhlmann, Moon Han, Jonathan S. Goldberg, Jeffrey Settleman, A. John Iafrate, Jeffrey A. Engelman, Daniel A. Haber, Bruce E. Johnson, and Thomas J. Lynch

Personalized Cancer Medicine

Clinical Molecular Profiling Core

CMPC Mission Statement

By developing and implementing state of the art genomic technologies, the Clinical Molecular Profiling Core will maximize the clinical benefits and biological insights derived from the analysis of biospecimens obtained from National Cancer Institute clinical trials.

More specifically, the CMPC seeks to aid investigators in:

- Tumor classifications and cancer gene discovery
- Discovery and validation of predictive and prognostic markers
- Pharmacodynamic marker discovery and monitoring
- Hypothesis based exploration of genes and molecular pathways
- Clinical correlation of research based observations

Clinical Molecular Profiling Core

Laboratory Organization

- Senior personnel: administrative, regulatory, scientific, statistical, pathology, protocol development

- Bench biologists: specimen processing, performing assays, technical development, some analysis

- Biostatisticians: analysis of data, suggestions for improved protocols

Clinical Molecular Profiling Core

NCI Clinical Protocols
Patient accrual and specimen collection

Senior Core personnel

NCI Principal investigators

CCR Tissue Procurement and Processing Facility (TPPF)
Accessioning and Specimen Tracking
Annotation

NCI support personnel

Clinical Molecular Profiling Core

Bench biologists

Bench biologists

Expression Profiling
mRNA
miRNA

Genomic Analysis
Array CGH
Methylation
DNA sequencing
SNP analysis

Tissue Analysis
TMA
IHC
LCM

Technical Development

Statisticians

Analysis

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Tools of the Trade

HumanRef-8 and HumanWG-6

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Clinical Emphasis

- Documented Training: assays, safety, procedures, ethics
- Standard Operating Procedures: specimen collection & processing, transport, assays, analysis, reporting
- Quality Control & Quality Assurance: temperatures, errors, instruments, assay controls, checklists

Clinical Molecular Profiling Core

Clinical Emphasis

- Federal Regulatory Requirements: Clinical Laboratory Improvement Act (CLIA;1988), NIH Guidelines

- Efficient Work Flows and Quality Results: automation, reproducible assays, identify checkpoints, and create an environment of excellence and improvement

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Clinical Collaborations

- MOB: 1) B-Raf & Ras mutations, 2) Thymoma
- Derm: 1) GvHD & 2) UV and drug effects
- Path: Prostate microenvironment using LCM
- Clin Immunotherapy: Hairy cell leukemia
- POB: Thyroid cancer
- ROB: Gastrointestinal cancer
- UOB: PRCC – MET mutations

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R&D Collaborations

- ATC: Angiogenesis Core
- SOB: Methylation in lung cancer cells
- Genetic Epi: RCC
- Lab Molecular Pharm: B-Raf mutations in cell line
- Pediatric Endocrinology: Glucocorticoid effects

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Workflow Issues

- Laboratory Forms
- Specimen QC
- Specimen Tracking
- Standard Operation Procedures

I. Laboratory Forms

How to address:

- Gathering of Information/Data
- Statement of Results
- Communication to PIs

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How to address: Gathering of Information/Data

Submit by Email | Print

CMPC
Clinical Molecular Profiling Core

Genetics Branch, CCR, NCI

CMPC Specimen Accessioning Form

NIH Study ID#:
Study Title:
Name of PI:
Cancer or Disease:
Specimen Type: Select from list or if not present, use custom entry
Fixative or stabilization method:
Number of specimens submitted:
Current storage conditions: Select from list
Requested date for results: or ☐ *None.*

Please note: an Excel/Word file with this information attached is also acceptable; electronic file attached?
☐ Yes ☐ No

Specimen Identifiers

1.	12.
2.	13.
3.	14.
4.	15.
5.	16.
6.	17.
7.	18.
8.	19.
9.	20.
10.	21.
11.	22.

(See next page for additional spaces)

Requested tests (please describe):

☐ Expression:	☐ Epigenetic:
☐ CGH:	☐ SNP:
☐ Sequencing:	☐ Other:

CMPC Specimen Accessioning Form (cont.)

NIH Study ID#:
Name of PI:

Specimen Identifiers

23.	44.
24.	45.
25.	46.
26.	47.
27.	48.
28.	49.
29.	50.
30.	51.
31.	52.
32.	53.
33.	54.
34.	55.
35.	56.
36.	57.
37.	58.
38.	59.
39.	60.
40.	61.
41.	62.
42.	63.
43.	64.

Note: If more than 64 specimens being submitted, please use a copy of this form.

Date received in CMPC: by: Select from list
Condition of specimens as received: Select from list
Has the PI provided H&Es of each specimen? ☐ Yes ☐ No
Has the specimen(s) been entered into Labmatrix? ☐ Yes ☐ No
Any quality control issues to report? ☐ No ☐ Yes. If Yes, please describe:

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How to address: Statement of Results

Submit by Email | Print Form

CMPC
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CMPC CGH Report Form

Report Date: ____________
Date Received: ____________
Study ID#: ____________
Study Name: ____________
Name of PI: ____________
CGH run(s) performed on: and ________ and ________ and ________
CMPC completion date: ____________
QC: Passed

Specimen ID#	CGH Test Utilized	Array ID#	Results	Interpretation
	☐Agilent aCGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	
	☐Agilent aCGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	
	☐Agilent aCGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	
	☐Agilent CGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	
	☐Agilent aCGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	

Report date: ____________
Study ID#:

Specimen ID#	CGH Test Utilized	Array ID#	Results	Interpretation
	☐Agilent aCGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	
	☐Agilent aCGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	
	☐Agilent aCGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	
	☐Agilent aCGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	
	☐Agilent aCGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	
	☐Agilent aCGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	
	☐Agilent aCGH ☐Illumina 317K SNP		☐No abnormalities detected ☐Abnormalities detected	

Comments: Detailed results to be sent under a separate cover.

______________________ __/__/__ or ______________________ __/__/__
Paul Meltzer, MD, PhD, Director   Daniel Edelman, PhD, Facility Head

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How to address: Communication to PIs

- Face-to-face meetings
- Emails with attachments
- FileMan web based program

II. Specimen QC

Establish QC criteria

- 260/280
- RIN scores
- H&E

Specimen labeling

Impact?

High Quality Results!

II. Specimen QC - **Papillary RCC Tissue**

PRCC samples tested for possible amplifications, especially of c-MET

Results:

Specimen: **204**
***Gain of 3, 7, 8, 16, 17*, 20**

Specimen: **455**
Loss of 3p (partial including VHL)

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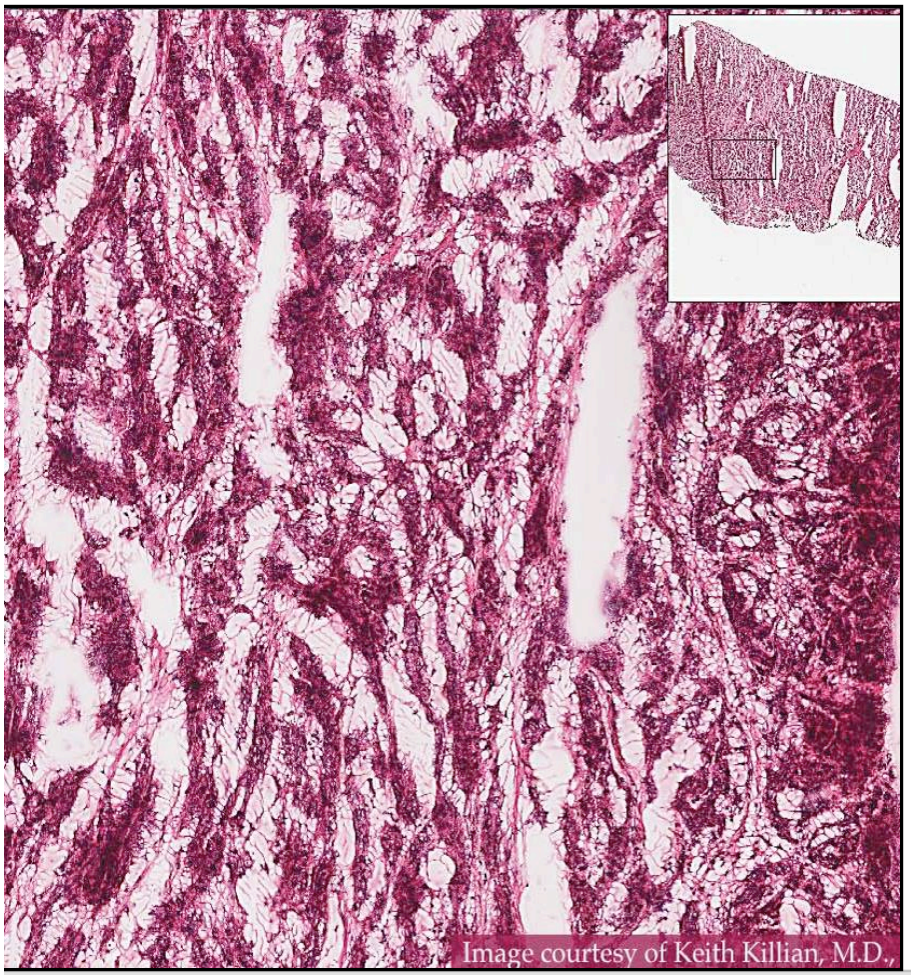
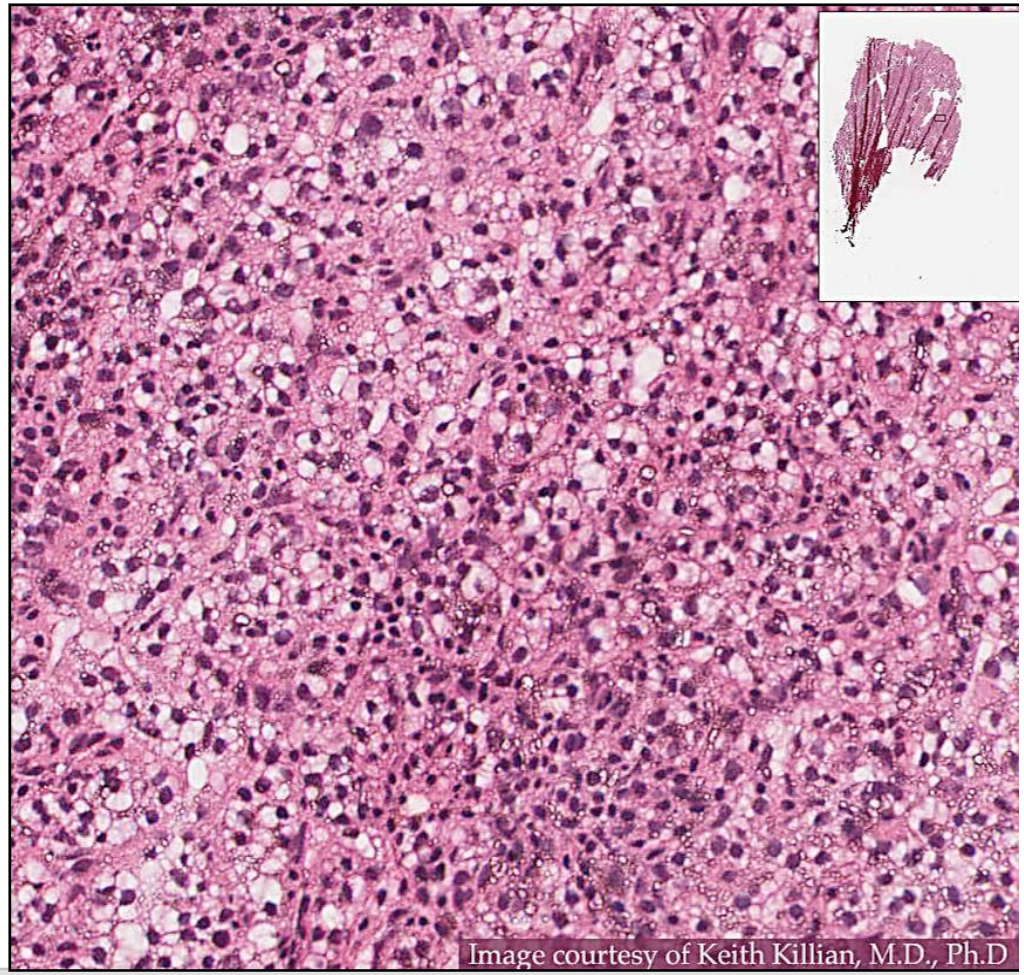
II. Specimen QC - **Papillary RCC Tissue**

#204



#455



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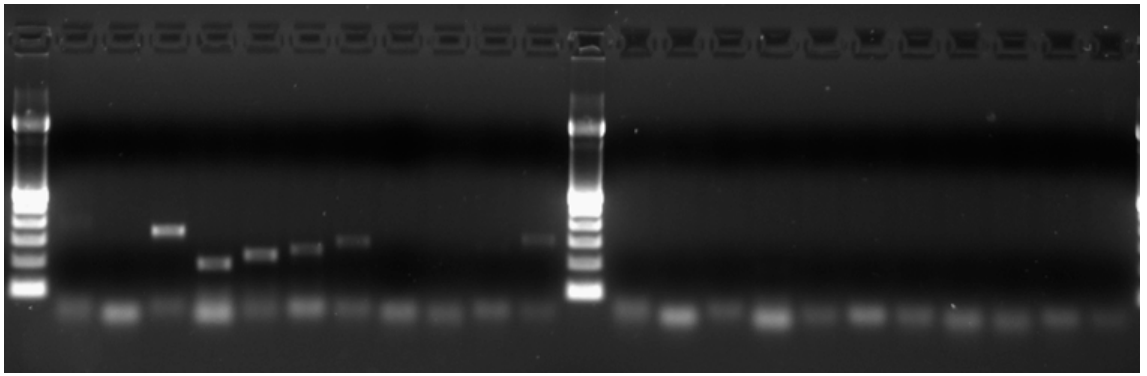
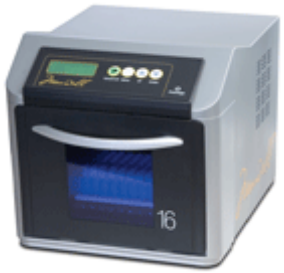
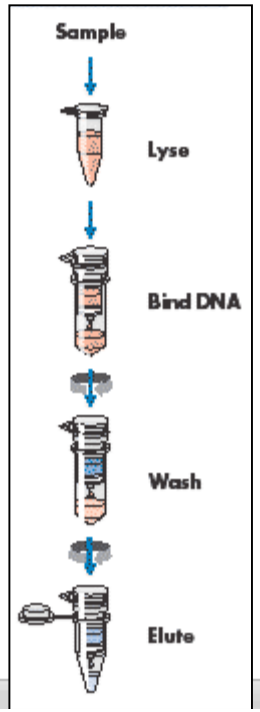
II. Specimen QC - **DNA Quality**

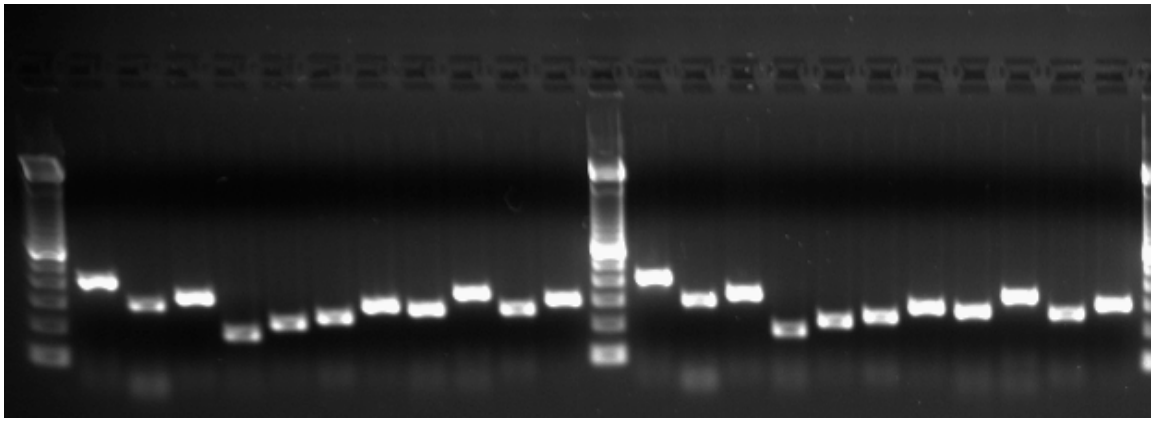
Promega Maxwell® 16 System







Qiagen DNA Micro Kit



III. Specimen Tracking

OHSR Sheet 14

NIH Requirements For The Research Use Of Stored Human Specimens And Data

The NIH IRB needs to consider "…a description of how the samples, specimens and/or data will be stored; ***how they will be tracked***;..."

DDIR Memorandum (June 12, 2006)

Research Use of Stored Human Samples, Specimens or Data

Such that "**NIH IRB-approved research protocols in which IRP researchers intend to collect and store human specimens or data:** …must include a written description of the intended use of the samples; how they will be stored; ***how they will be tracked***;…". "…***consistent with DHHS requirements***."

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III. Specimen Tracking - Labmatrix

Labmatrix - Mozilla Firefox

File Edit View History Bookr

Labmatrix

Subjects Contacts Communications Biomaterials Genotypes Storage Custom Data Workflow Query Log Off Imports Admin Help

Location: Meltzer ATC Freezer #5

Container	Contents
Meltzer ATC Freezer #5	
A - Freezer Box	
B - Row B	
C - Temp Pan	
D	
E	

Favorites Recent Biomaterial Search Results

Name	Class	Type	Barcode	Subject Prim...	Subject Code	Subject Custom ID
HV01- Ex'	Biomolecule	RNA	HV01- Ex'	Skin PT Study	Skin PT Study.1	HV01
HV01- Unex'	Biomolecule	RNA	HV01- Unex'	Skin PT Study	Skin PT Study.1	HV01
HV01-D-Ex 1:35	Biomolecule	RNA	HV01-D-Ex 1:35	Skin PT Study	Skin PT Study.1	HV01
HV01-D-Unex 1:40	Biomolecule	RNA	HV01-D-Unex ...	Skin PT Study	Skin PT Study.1	HV01
HV02- Ex 9:22	Biomolecule	RNA	HV02- Ex 9:22	Skin PT Study	Skin PT Study.2	HV02
HV02- Unex 9:26	Biomolecule	RNA	HV02- Unex 9:26	Skin PT Study	Skin PT Study.2	HV02
HV02-D-Ex 9:27	Biomolecule	RNA	HV02-D-Ex 9:27	Skin PT Study	Skin PT Study.2	HV02
HV02-D-Unex 9:30	Biomolecule	RNA	HV02-D-Unex ...	Skin PT Study	Skin PT Study.2	HV02
HV04- Ex 3:15	Biomolecule	RNA	HV04- Ex 3:15	Skin PT Study	Skin PT Study.3	HV04
HV04- Unex 3:21	Biomolecule	RNA	HV04- Unex 3:21	Skin PT Study	Skin PT Study.3	HV04

Records 1-128 of 128 total found Print Refresh

67725 HV01- Ex' 69884 Biomolecule 69884 Workspace

Create View Print Delete Edit

Biomolecule - 69884 Biomolecule 69884

Biomaterial Lineage Custom Data Genotypes Attachments

- Skin PT Study.1 (HV01)
  - 67725 HV01- Ex'
    - 69884 Biomolecule 69884
  - 67726 HV01- Unex'
    - 70309 cDNA 70309

Print Refresh

Created: 5/13/2008 3:15 PM Modified: 5/19/2008 1:17 PM

Configurations

- 3 tier dwr rack
- 36 vial box
- 5x5 box
- 81
- box rack

IV. Standard Operating Procedures

Elements there of:

- Purpose/Introduction
- Workflow chart
- Principle
- Equipment
- Reagents & Supplies
- Protocol
- Troubleshooting
- Quality Control
- Tracking sheet

IV. Standard Operating Procedures

Examples there of:

- Specimen collection and storage
- Specimen accessioning
- Specimen processing
- Specimen tracking
- Shipping
- Specimen workflow
- Test procedures
- Result reporting
- …and more.

Impact:

- Reproducible results
- Quality results
- Assists in training
- Meet regulatory requirements
- …and more!

Tech Development
RNA Extraction

Purpose:

To investigate and validate a non-organic extraction method for *TOTAL* RNA that 1) obviates the need for organics, 2) provides for high throughput processing, and 3) extracts small RNAs.

Methods:

Cell line: A549 (Human lung adenocarcinoma epithelial)

Reagents:

1) Trizol (organic)
2) Qiagen (non-organic; modified protocol)
   - RNeasy Plus Mini Kit
   - gDNA Eliminator Mini SpinColumns
   - QIAshredder - disposable cell-lysate homogenizers
3) AquaRNA (non-organic; MultiTarget Pharmaceuticals LLC)

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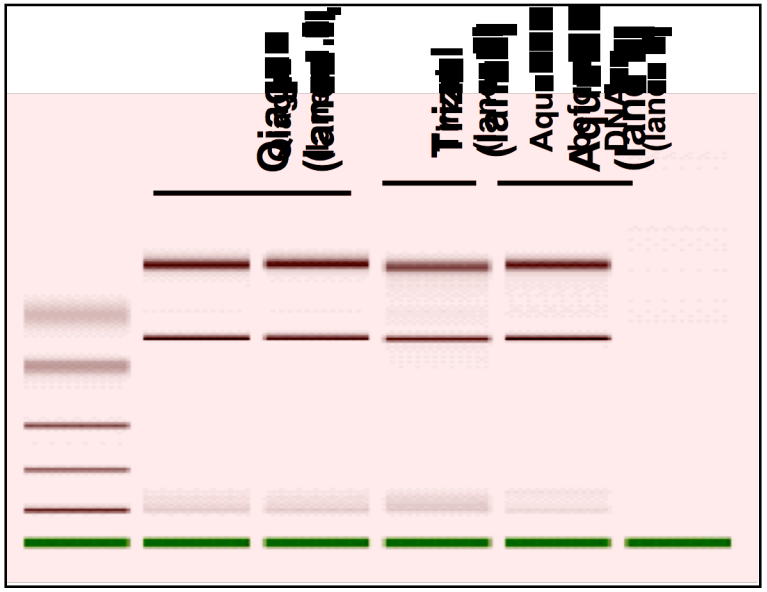
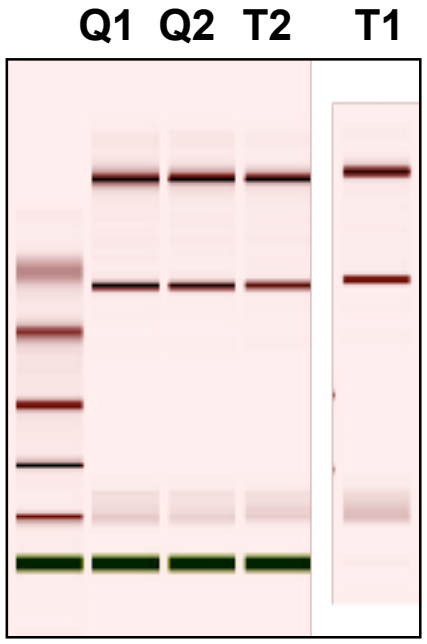
RNA Extraction

Results: Total RNA (Bioanalyzer)





	Qiagen1 and Q2	Trizol1 and T2
Total RNA per cell prep recovered	38ug and 35ug **	28ug and 34ug **
Nanodrop	1.2ug/ul and 1.1ug/ul	1.1ug/ul and 2.6ug/ul
260/280	2.0 and 2.0	1.96 and 1.98
BioAnalzer conc	1.0ug/ul and 0.9ug/ul	1.7ug/ul and 0.84ug/ul
RIN#	9.9 and 10	10 and T2=not determined

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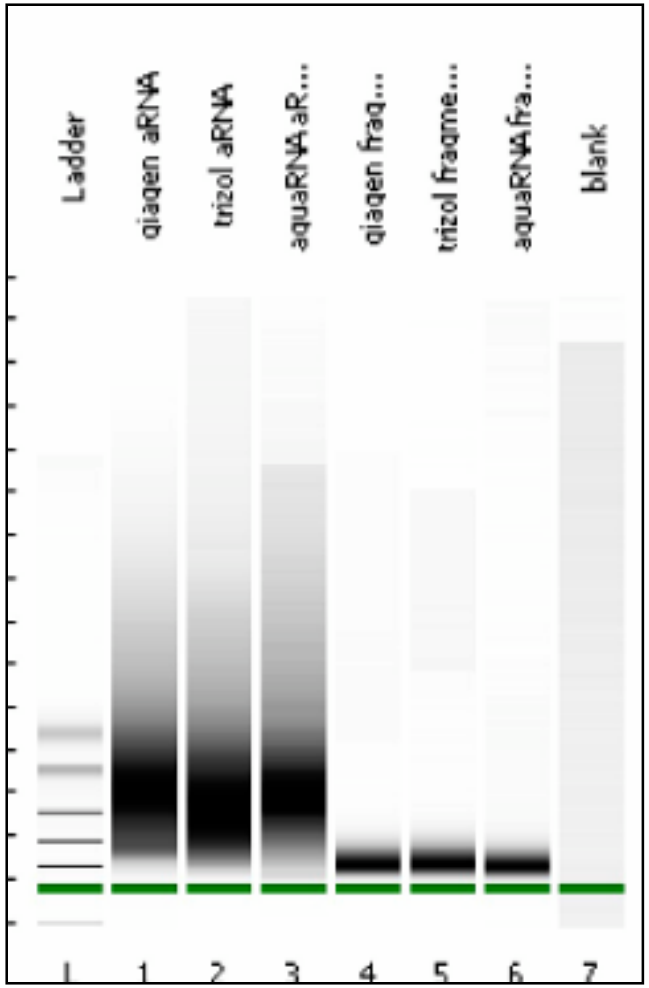
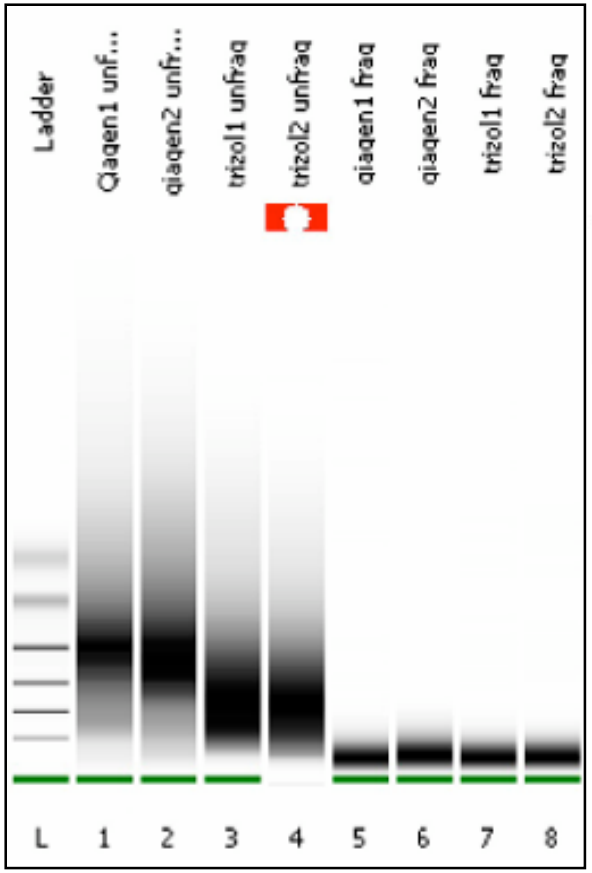
RNA Extraction

Results:

aRNA & Fragmentation





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RNA Extraction

Results: Affymetrics

	Qiagen	Trizol	AquaRNA
microarray results			
Scale factor %genes presen			
Correlation plo (see "affy worksh			

	Qiagen		Trizol
microarray results			
Scale factor %genes present***	1.5 and 1.1 48% and 46.8%		4.6 and 3.4 40% and 43%
Correlation plots (see "affy worksheet")	Qiagen 1 vs Qiagen 2	92%	
	Trizol 1 vs Trizol 2	97%	
	Qiagen 1 vs Trizol 1	83%	

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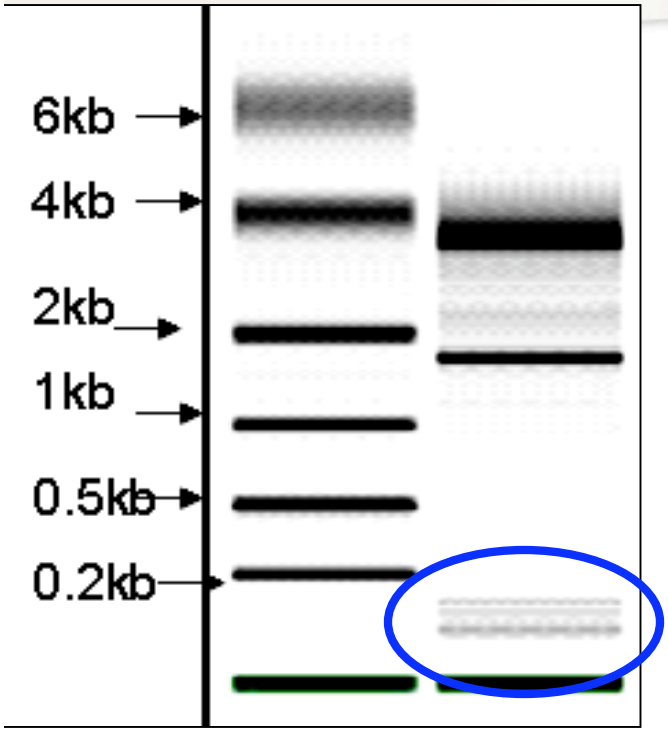
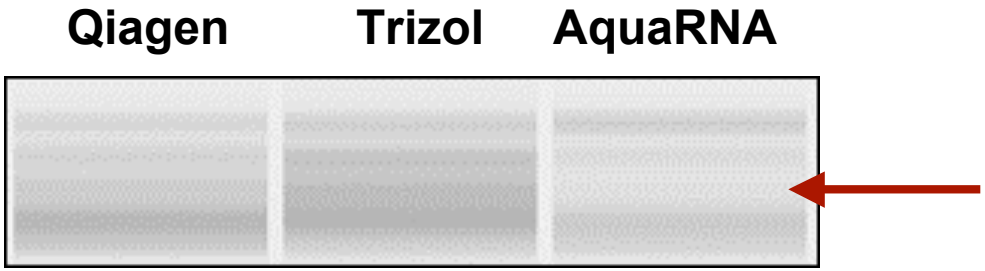
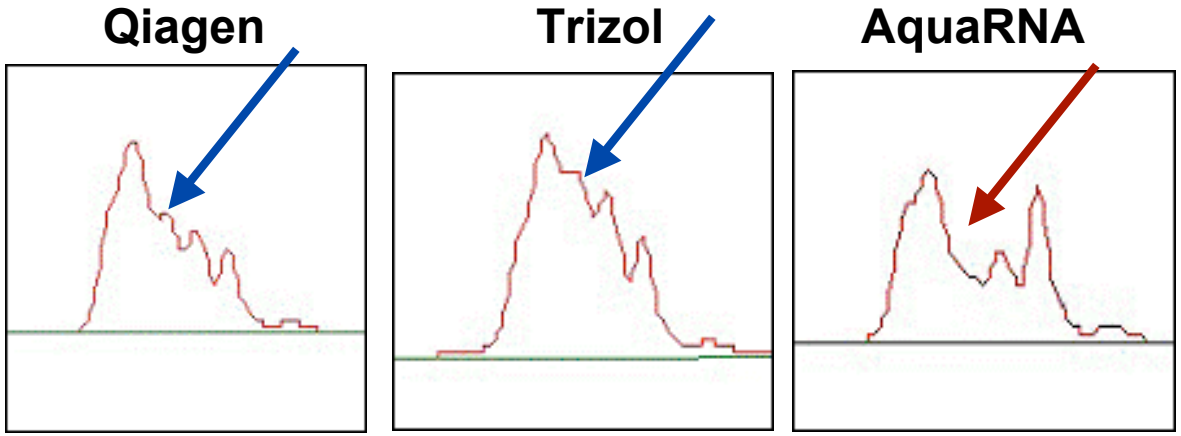


RNA Extraction

Small RNAs





RNA Extraction

Small and microRNAs:

Method: QPCR using primer sets from Qiagen

- let-7a
- miR-16
- miR-21
- RNU6B

Corbett Rotorgene thermocycler

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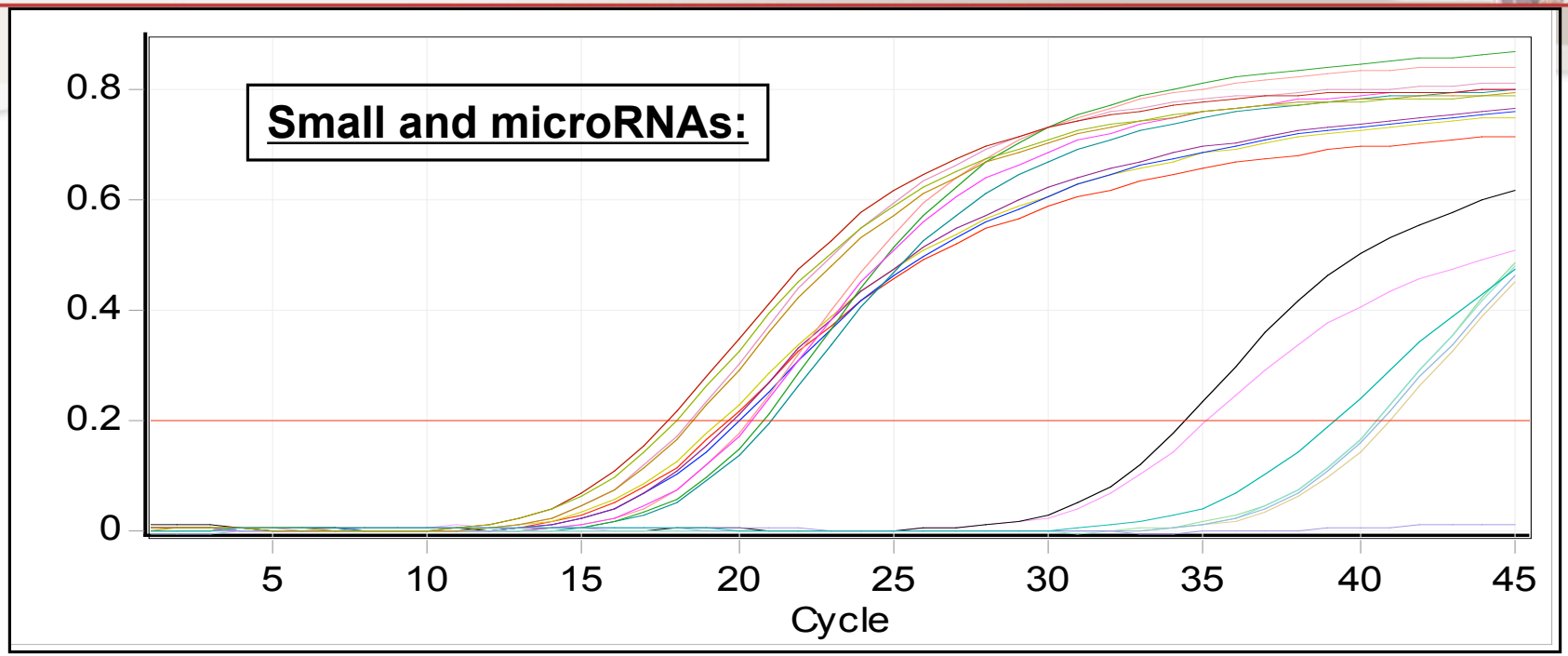

	Cts			
Name	let-7a	miR-16	miR-21	RNU6B
Trizol 1	**19.68**	**21.03**	***40.98***	**18.41**
Trizol 2	**19.42**	**20.34**	***40.51***	**17.73**
Qiagen 1	**20.02**	**20.78**	***40.52***	**18.52**
Qiagen 2	**19.77**	**20.39**	***40.71***	**17.95**
NT	***35.1***	***34.4***		***39.16***

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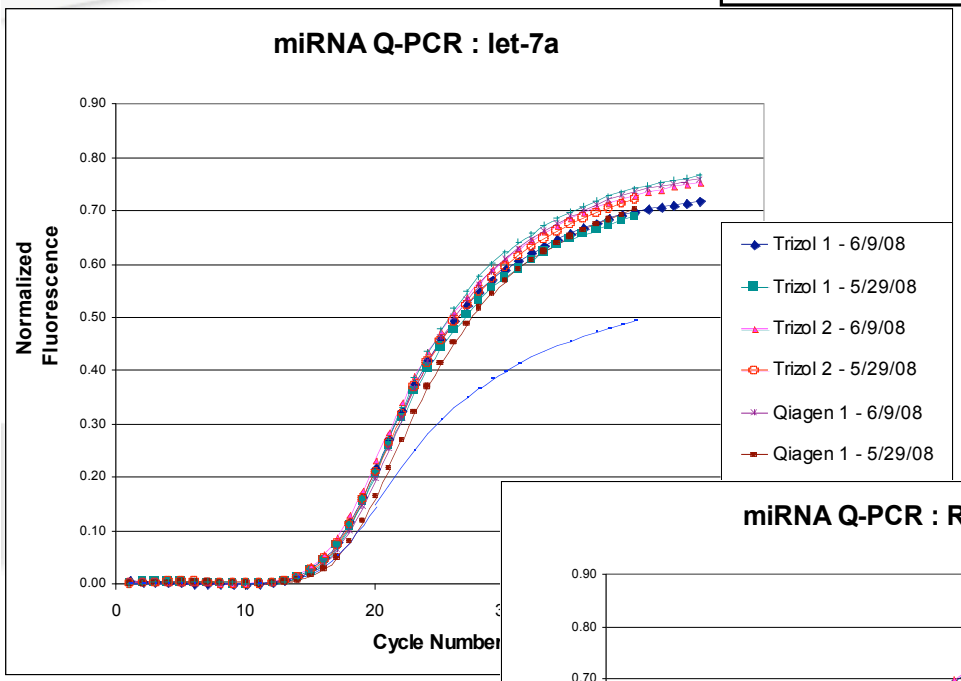
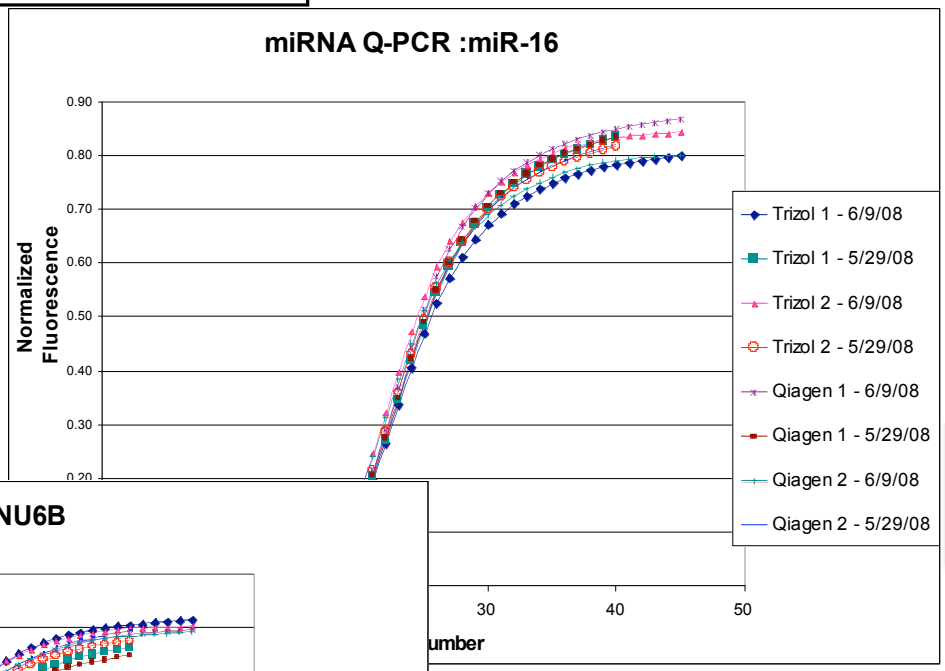
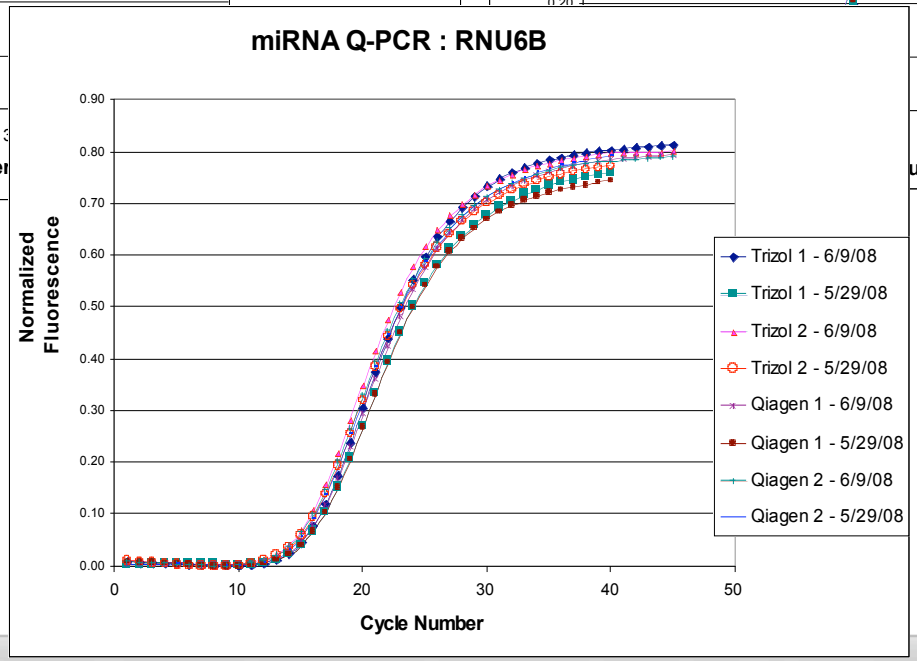
Small and microRNAs:







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Small and microRNAs:

	Cts			ΔCt (miRNA - RNU6B)	
	let-7a	miR-16	RNU6B	let-7a	miR-16
Trizol 1 - 6/9/08	19.68	21.03	18.41	1.27	2.62
Trizol 1 - 5/29/08	19.86	20.97	18.84	1.02	2.13
Trizol 2 - 6/9/08	19.42	20.34	17.73	1.69	2.61
Trizol 2 - 5/29/08	19.79	20.77	18.1	1.69	2.67
Qiagen 1 - 6/9/08	20.02	20.78	18.52	1.50	2.26
Qiagen 1 - 5/29/08	20.67	20.92	18.87	1.80	2.05
Qiagen 2 - 6/9/08	19.77	20.39	17.95	1.82	2.44
Qiagen 2 - 5/29/08	21.47	20.83	18.13	3.34	2.70

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Small and microRNAs:

And what about miR-21???

nature biotechnology

MicroRNAs accurately identify cancer tissue origin

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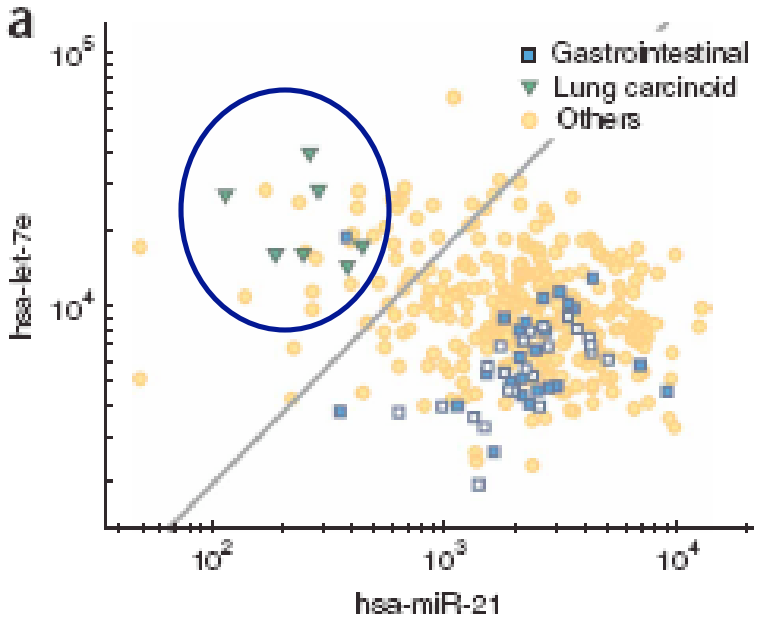
Figure 2



Clinical Molecular Profiling Core

RNA Extraction

Conclusion:

- Comparable concentrations and yields
- Comparable RIN numbers & 260/280
- Comparable detection of gene expression
- Both the Trizol and Qiagen methods result in what appear to be small molecular RNAs

Decision:

Either method (T or Q) is suitable; however, Qiagen was chosen to replace Trizol due to:

- ✓ Comparable results to standard
- ✓ Ease of use
- ✓ Possibility of automation
- ✓ Established chemistry and technology
- ✓ NO HAZARDOUS WASTE

Future Directions

CMPC oriented

- CLIA certification
- Continually develop and refine SOPs
- Improve specimen and testing workflows
- Improved TAT
- Expanded training
- Raise QC awareness

Future Directions

Collaboration oriented

- Outreach presentations
- More involved in protocol development
- Increase awareness of collaborative activities
- Increase involvement in follow up and validation studies

Clinical Molecular Profiling Core

Acknowledgements & Personnel

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